**We claim:**

1. A method of preparing a maltogenic amylase variant having improved anti-staling properties, which method comprises

a) subjecting a DNA sequence encoding the maltogenic amylase to random mutagenesis,

b) expressing the mutated DNA sequence obtained in step (a) in a host cell, and

c) screening for host cells expressing a mutated maltogenic amylase which shows a higher thermostability, and

d) preparing the mutated maltogenic amylase expressed by the host cells.

2. The method of claim 1, wherein the mutated DNA sequence is expressed by transforming a suitable host cell with the mutated DNA sequence and culturing the host cell obtained in step (b) under suitable conditions for expressing the mutated DNA sequence.

3. A method of producing a variant of a parent maltogenic alpha-amylase, said method comprising

a) modeling the parent alpha-amylase on the three-dimensional structure of SEQ ID NO: 1 depicted in the Appendix to produce a three-dimensional structure of the parent alpha-amylase;

b) identifying in the three-dimensional structure obtained in step (a) at least one structural part of the parent wherein an alteration in said structural part is predicted to result in said altered property;

c) modifying the sequence of a nucleic acid encoding the parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said structural part; and

d) expressing the modified nucleic acid in a host cell to produce the variant alpha-amylase,

wherein the variant has alpha-amylase enzymatic activity and has at least one altered property relative to the parent.

4. The method of claim 3, wherein the altered property is pH dependent activity, thermostability, substrate cleavage pattern, specific activity of cleavage, transglycosylation, ability to reduce retrogradation of starch, ability to reduce staling of bread, substrate specificity, substrate binding or calcium binding.

5. A method of constructing a variant of a parent maltogenic alpha-amylase, which method comprises:

a) identifying an amino acid residue which is within 15 Å (in particular 10 Å) from an active site residue of the parent amylase in the three-dimensional structure of said parent, and which is involved in electrostatic or hydrophobic interactions with an active site residue;

b) substituting said amino acid residue with another amino acid residue which changes the electrostatic and/or hydrophobic surroundings of an active site residue, and which can be accommodated in the structure;

c) optionally repeating steps a) and b) recursively;

d) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than b),

e) preparing the variant resulting from steps a) - d);

f) testing the pH dependent activity of said variant; and

g) optionally repeating steps a) - f) recursively; and

h) selecting a variant having an altered pH dependent activity as compared to the parent amylase.

6. A method of constructing a variant of a parent maltogenic alpha-amylase, which method comprises:

a) identifying an internal cavity or crevice in the three-dimensional structure of said parent;

b) substituting an amino acid residue in the neighborhood of the cavity or crevice with another amino acid residue which increases the hydrophobic interaction and/or fills out or reduces the size of the cavity or crevice;

c) optionally repeating steps a) and b) recursively;

d) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than b),

e) preparing the variant resulting from steps a) - d);

f) testing the thermostability of said variant; and

g) optionally repeating steps a) - f) recursively; and

h) selecting a variant having increased thermostability as compared to the parent amylase.

7. The method of claim 6, wherein the substitution of the amino acid residue results in increasing the hydrophobic interaction, substitution with proline, substitution of histidine with another amino acid, stabilization of calcium binding, introduction of an interdomain disulfide bond, removal of a deamidation site, altering a hydrogen bond contact, filling in an internal structural cavity with an amino acid with a bulkier side group, introduction of interdomain interactions, altering charge distribution, helix capping, or introduction of a salt bridge.

8. A method of constructing a variant of a parent maltogenic alpha-amylase, which method comprises:

a) identifying an amino acid residue within 10 Å from a calcium binding site in the three dimensional structure of the amylase;

b) substituting the amino acid residue with another amino acid residue so as to improve the interaction with the calcium ion;

c) optionally repeating steps a) and b) recursively;

d) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than b),

e) preparing the variant resulting from steps a) - d);

f) testing the thermostability of said variant; and

g) optionally repeating steps a) - f) recursively; and

h) selecting a variant having increased thermostability as compared to the parent amylase.

9. A method of constructing a variant of a parent maltogenic alpha-amylase, which method comprises:

a) identifying the substrate binding area in a model of the three-dimensional structure of the parent amylase;

b) modifying the substrate binding area by an amino acid substitution, deletion or insertion;

c) optionally repeating step b) recursively;

d) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than b),

e) preparing the variant resulting from steps a) - d);

f) testing the substrate-cleavage pattern of the variant.

g) optionally repeating steps a) - f) recursively; and

h) selecting a variant having an altered substrate-cleavage pattern as compared to the parent amylase.

10. A polypeptide which:

a) has maltogenic amylase activity;

b) has at least 70 % identity to SEQ ID NO: 1,

c) has optimum maltogenic amylase activity in the range pH 3.5-7.0 (preferably 4-5.5), and

d) shows a residual maltogenic amylase activity of at least 25 % after incubation with 1 mM $Ca^{++}$ at pH 4.3, 80°C for 15 minutes.

11. A polypeptide which:

a) has maltogenic alpha-amylase activity;

b) has at least 70 % identity to SEQ ID NO: 1; and

c) comprises an amino acid modification compared to SEQ ID NO: 1 at a position corresponding to Q13, I16, D17, N26, N28, P29, A30, S32, Y33, G34, L35, K40, M45, P73, V74, D76, N77, D79, N86, R95, N99, I100, H103, Q119, N120, N131, S141, T142, A148, N152, A163, H169, N171, G172, I174, N176, N187, F188, A192, Q201, N203, H220, N234, G236, Q247, K249, D261, N266, L268, R272, N275, N276, V279, N280, V281, D285, N287, F297, Q299, N305, K316, N320, L321, N327, A341, N342, A348, Q365, N371, N375, M378, G397, A381, F389, N401, A403, K425, N436, S442, N454, N468, N474, S479, A483, A486, V487, S493, T494, S495, A496, S497, A498, Q500, N507, I510, N513, K520, Q526, A555, A564, S573, N575, Q581, S583, F586, K589, N595, G618, N621, Q624, A629, F636, K645, N664 and/or T681; and

d) has improved stability compared to the polypeptide of SEQ ID NO: 1.

12. The polypeptide of claim 11, wherein the modification comprises an amino acid modification at a position corresponding to K40, V74, H103, S141, T142, F188, H220, N234, K249, D261, L268, V279, N342, H344, G397, A403, K425, S442, S479, S493, T494, S495, A496, S497, A498, Q500, K520, A555 and/or N595; preferably a substitution corresponding to K40R, V74P, H103Y/V/I/L/F/Y, S141P, T142A, F188I/L, H220Y/L/M, N234P, K249P, D261G, L268P, V279P, N342P, H344E/Q/N/D/Y, G397P, A403P, K425E, S442P, S479P, S493P, T494P, S495P, A496P, S497P, A498P, Q500P, K520R, A555P and/or N595I.

13. The polypeptide of claim 11 or 12, wherein the modification comprises an amino acid modification at a position corresponding to D17, N28, P29, A30, S32, Y33, G34, R95, H103, N131, H169, I174 and/or Q201 such as to improve calcium coordination, preferably a substitution corresponding to D17Q/E, A30D/M/L/A/V/I/E/Q, S32D/E/N/Q, R95M/L/A/V/I/E/Q, H103Y/N/Q/D/E, N131D, H169N/D/E/Q, I174E/Q, Q201E.

14. The polypeptide of any of claims 11-13, wherein the modification comprises a substitution at a position corresponding to Q13, N26, N77, N86, N99, Q119, N120, N131, N152, N171, N176, N187, Q201, N203, N234, Q247, N266, N275, N276, N280, N287, Q299, N320, N327, N342, Q365, N371, N375, N401, N436, N454, N468, N474, Q500, N507, N513, Q526, N575, Q581, N621, Q624 and/or N664 such as to remove a deamidation site, preferably a substitution corresponding to Q13S/T/A/V/L/I/F/M, N26S/T/A/V/L/I, N77S/T/A/V/L/I, N86S/T/A/V/L/I, N99T/S/V/L, Q119T/S, N120S/T/A/V/L/I, N131S/T/A/V/L/I, N152T/S/V/L, N171Y/D/S/T, N176S/T/A/V/L/I, N187S/T/A/V/L/I, Q201S/T/A/V/L/I/F/M, N203D/S/T/A/V/L/I, N234S/T/A/V/L/I, Q247S/T/A/V/L/I/F/M, N266S/T/A/V/L/I, N275S/T/A/V/L/I, N276S/T/A/V/L/I, N280S/T/A/V/L/I, N287S/T/A/V/L/I, Q299L/T/S, N320S/T/A/V/L/I, N327S/T/A/V/L/I, N342S/T/A/V/L/I, Q365S/T/A/V/L/I, N371S/T/A/V/L/I, N375S/T/A/V/L/I, N401S/T/A/V/L/I, N436S/T/A/V/L/I, N454D/S/T/A/V/L/I, N468D/S/T/A/V/L/I, N474D/S/T/A/V/L/I, Q500S/T/A/V/L/I/F/M, N507S/T/A/V/L/I, N513S/T/A/V/L/I, Q526 D/S/T/A/V/L/I, N575S/T/A/V/L/I, Q581S/T/A/V/L/I/F/M, N621S/T/A/V/L/I Q624S/T/A/V/L/I/F/M and/or N664D/S/T/A/V/L/I.

15. The polypeptide of any of claims 11-14, wherein the modification comprises a substitution at a position corresponding to I16, L35, M45, P73, D76, D79, A192, I100, A148, A163+G172, L268, V281, D285, L321, F297, N305, K316, S573, A341, M378, A381, F389, A483, A486, I510, A564, F586, K589, F636, K645, A629, and/or T681 such as to improve hydrogen bond contact, preferably a substitution corresponding to I16T/D/N, L35Q, M45K, P73Q, D76E, D79E/Y, A192S/D/N, I100T/S/D/N/E/Q, A148D/N/E/Q/S/T/R/K, A163Y+G172S/D/N, L268R/K, V281/Q, D285R/K, L321Q, F297N/D/Q/E, N305K/R, K316N/D, S573N/D, A341R/K, M378R/K, A381S/D/N, F389Y, A483S/D/N, A486Q/E, I510R/K, A564S/D/N, F586S/D/N, K589S/D/Q/N, F636Y, K645T, A629N/D/E/Q, and/or T681D/N/E/Q/S.

16. The polypeptide of any of claims 11-15, wherein the modification comprises substitutions such as to introduce one or more interdomain disulfide bonds, preferably corresponding to G236C + S583C, G618C + R272C, and/or A348C + V487C.

17. The polypeptide of any of claims 11-16, wherein the substitution at a position corresponding to L51, L75, L78, G88, G91, T94, V114, I125, V126, T134, G157, L217, S235, G236, V254, V279, V281, L286, V289, I290, V308, L321, I325, D326, L343, F349, S353, I359, I405, L448, Q449, L452, I470, G509, V515, S583, G625, L627, L628 and/or A670 so as to fill an internal cavity or crevice, preferably a substitution corresponding to L51W, L75F/Y, L78I, G88A/V/T, G91T/S/V/N, T94V/I/L, V114V/I/L, I125L/M/F/Y/W, V126I/L, T134V/I/L/M/F/Y/W, G157A/V/I/L,

L217V/I/M/F/Y/W, S235I/L/M/F/Y/W, G236A/V/I/L/M/F/Y/W, V254I/L/M/F/Y/W, V279M/I/L/F, V281I/L/M/F/Y/W, L286F, V289I/L/R, I290M/L/F, V308I/L/M/F/Y/W, L321I/M/F/Y/W, I325L/M/F/Y/W, D326E/Q, L343M/F/Y/W, F349W/Y, S353V/I/L, I359L/M/F/Y/W, I405M/L/Y/F/W, L448Y, Q449Y, L452M/Y/F/W, I470M/L/F, G509A/V/I/L/M/S/T/D/N, V515I/L, S583V/I/L/V, G625A/V/I/L/M/F/Y/W, L627M/F/Y, L628M/I/F/Y/W, A670V/I/L/M/F/Y/W and/or L217 in combination with L75 (e.g. L217F/Y in combination with L75F/Y).

18. The polypeptide of any of claims 11-17, wherein the modification comprises a substitution at a position corresponding to N106, N320 and Q624 so as to create a salt bridge, preferably a substitution corresponding to N106R, N320E/D and/or Q624E.

19. The polypeptide of any of claims 11-18, wherein the modification comprises a substitution at a position corresponding to K244 and/or K316 such as to alter the charge distribution, preferably a substitution corresponding to K244S and/or K316G/N/D.

20. The polypeptide of any of claims 11-19, wherein the modification comprises a substitution at a position corresponding to V281 and/or A629 such as to alter the binding site, preferably a substitution corresponding to V281Q and/or A629N/D/E/Q.

21. The polypeptide of any of claims 11-20, wherein the modification comprises substitutions such as to alter the interdomain interaction at a position corresponding to F143+F194+L78, A341+A348+L398+I415+T439+L464+L465, L557, S240+L268, Q208+L628, F427+Q500+N507+M508+S573 and/or I510+V620, preferably substitutions corresponding to F143Y+F194Y+L78Y/F/W/E/Q, A341S/D/N+A348V/I/L+L398E/Q/N/D+I415E/Q+T439D/E/Q/N+L464D/E+L465D/E/N/Q/R/K, L557Q/E/N/D, S240D/E/N/Q+L268D/E/N/Q/R/K, Q208D/E/Q+L628E/Q/N/D, F427E/Q/R/K/Y+Q500Y+N507Q/E/D+M508K/R/E/Q+S573D/E/N/Q; and/or I510D/E/N/Q/S+V620D/E/N/Q.

22. A polypeptide which:

a) has maltogenic alpha-amylase activity;

b) has at least 70 % identity to SEQ ID NO: 1;

c) comprises an amino acid modification compared to SEQ ID NO: 1 at a position corresponding to D127, V129, F188, A229, Y258, V281, F284, T288, N327, M330, G370, N371, and/or D372; and

d) has altered pH dependent activity as compared to the polypeptide of SEQ ID NO: 1.

23. The polypeptide of claim 22, wherein the modification comprises a substitution corresponding to D127N/L, V129S/T/G/V, F188E/K/H, A229S/T/G/V, Y258E/D/K/R/F/N, V281L/T, F284K/H/D/E/Y, T288E/K/R, N327D, M330L/F/I/D/E/K, G370N, N371D/E/G/K, and/or D372N/V.

24. A polypeptide which:

a) has maltogenic alpha-amylase activity;

b) has at least 70 % identity to SEQ ID NO: 1;

c) comprises an amino acid modification compared to SEQ ID NO: 1 at a position corresponding to P191, A192, G193, F194 and/or S195; and

d) has higher specific amylase activity than the polypeptide of SEQ ID NO: 1.

25. The polypeptide of claim 24, wherein the modification comprises a deletion, preferably the deletion Δ (191-195).

26. The polypeptide of claim 24, wherein the modification comprises insertion, preferably 192-A-193.

27. A polypeptide which:

a) has maltogenic alpha-amylase activity;

b) has at least 70 % identity to SEQ ID NO: 1;

c) comprises an amino acid modification compared to SEQ ID NO: 1 at a position corresponding to A30, K40, N115, T142, F188, T189, P191, A192, G193, F194, S195, D261, T288, N327, K425, K520 and/or N595; and

d) has a higher ability than the polypeptide of SEQ ID NO: 1 to reduce retrogradation of starch and/or staling of bread.

28. The polypeptide of claim 27, wherein the modification comprises A30D, K40R, N115D, T142A F188L, T189Y, Δ (191-195), D261G, T288P, N327S, K425E, K520R and/or N595I.

29. A process for preparing a dough or a baked product prepared from the dough which comprises adding the polypeptide of any of claims 10-28, or a variant produced by the method of any of claims 1-8 to the dough in an amount which is effective to retard the staling of the bread.

30. The process of claim 29, wherein the variant is added in an amount of 0.1-5 mg per kg of flour, preferably 0.5-2 mg/kg.

31. A nucleic acid sequence encoding the polypeptide of any of claims 10-27, preferably operably linked to one or more control sequences which direct the expression of the variant in a suitable expression host.

32. A recombinant expression vector comprising the nucleic acid sequence of claim 31, a promoter, and transcriptional and translational stop signals, and preferably further comprising a selectable marker.

33. A transformed host cell comprising the nucleic acid sequence of claim 31 or the vector of claim 32.

34. A method for producing the polypeptide of any of claims 10-27, comprising:

a) cultivating the transformed host cell of claim 33 under conditions conducive to expression of the variant; and

b) recovering the variant.

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